5. Smith, A.D.M. (2013). Fishery management: is Europe turning the corner? Curr. Biol. *23*, R661–R662.
6. Lund, J., and Gabrielsen, V. (2005). A Fishy Business. Transport Amphorae of the Black Sea Region as a Source of the Trade in Fish and Fish Products in the Classical and Hellenistic Periods. Ancient Fishing and Fish Processing in the Black Sea Region (Gylling: Aarhus University Press), pp. 161–169.
7. FAO (2003). Fisheries management. 2. The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries *4* (suppl. 2), 112.
8. The State of World Fisheries and Aquaculture (2014). Opportunities and Challenges (Rome: FAO), p. 223.
9. Worm, B., and Branch, T. (2012). The future of fish. Trends Ecol. Evol. *27*, 594–599.
10. European Communities (2004). Proceedings of the Third Ministerial Conference on the Sustainable Development of Fisheries in the Mediterranean. Venice, Italy, 25–26 November 2003.

[1]CSIRO Oceans and Atmosphere Flagship, Hobart, Tasmania, Australia. [2]Chair of the Fisheries Expert Group of the IUCN Commission on Ecosystem Management (IUCN-CEM-FEG), Brussels, Belgium.
*E-mail: Tony.d.smith@csiro.au

http://dx.doi.org/10.1016/j.cub.2014.07.031

Neurogenetics: Sex and the Female Brain

Male flies put on a multimedia show during courtship involving dance, song, perfume and even vibrations; if a female likes it, she pauses to let him know. Recent studies shed new light on how development and experience contribute to neural mechanisms of female sexual receptivity.

Jean-Christophe Billeter[1] and Joel D. Levine[2]

Mechanisms of sensory-motor integration during social interactions are well illustrated in the sexual interactions of the fruit fly *Drosophila melanogaster*. Thanks to precision of *Drosophila* genetics, the neuronal and molecular substrates that permit males to sense and court females are being dissected down to the single neuron [1]. But what about females? We lack knowledge of how females sense, interpret, and respond to the information communicated by males through courtship. Three new studies [2–4] have now uncovered genes and neurons that control female receptivity during her response to male courtship, providing a glimpse of the developmental origin and physiology of the neuronal circuitry behind female mating decisions.

There is a great deal to be learned from understanding the female nervous system and female behavior. In her essay *A Room of One's Own*, Virginia Woolf once asked "Why are women... so much more interesting to men than men are to women?" Because of the evolutionary function of females as gatekeepers of gene flow between species and because females select males whose genes are best fitted for a given environment, one can expect that the study of female mating decisions will ultimately uncover more complex mechanisms than that of males. The more nuanced role of the female is certainly of great interest to males and based on these studies may end up more interesting to us all.

As young adults, female flies are unreceptive to male advances. But once hormones kick in and females become sexually mature, they demand an exacting courtship performance: the male runs after a female while extending and vibrating one wing to produce a song; he displays chemical tastes and odors meticulously synthesized in his body; and his abdomen quivers, sending vibrations through the physical substrate beneath them [5,6]. If satisfied with this multimedia display, the female will slow down and allow the male to mount her. Once the male has dismounted her, that same female will become even pickier and vehemently rejects the advances of new suitors by sticking out her ovipositor in their faces, or flicking them off, a condition called unreceptive post-mating state (reviewed in [5]).

Historically, female receptivity has been difficult to assay because females are conspicuously passive during courtship, making genetic analysis difficult [5]. In a study from the Vosshall lab published recently in *Current Biology*, Bussell *et al.* [2] report a virgin female behavior they call 'pausing'. Computer-mediated tracking of movement during courtship shows that females pause movements intermittently. Although weakly correlated in time with bouts of male singing, pausing correlates strongly with female receptivity. That female pausing is connected to male courtship song is indicated by the observation that pausing decreases when a male lacks wings (rendering him unable to produce a courtship song) and increases when the song is played back.

A similar stopping of female movement had been previously reported in *Current Biology* by Fabre *et al.* [6], who showed that periods of immobility correlated much more precisely with another male behavior than with song: that is, when the male quivers his abdomen to produce substrate borne vibrations. Several male signals therefore feed into the female decision to pause, probably also including male pheromones, another important determinant of female mate choice [7]. The speed of female movement had previously been shown to affect male courtship style [8], indicating that pausing may provide males feedback about their performance rather than permission to copulate. This is consistent with the observation that males attempt to copulate even when pausing is reduced or fails to increase [2].

The genetic basis of pausing provides an interesting lesson about how behaviors are controlled. Following a genome-wide screen for neuronally expressed genes necessary for receptivity, Bussell *et al.* [2] demonstrate that the homeotic gene *Abdominal-B* (*Abd-B*) is required in the female nervous system to control pausing and receptivity. Their experiments indicate that *Abd-B* functions only during development to control these phenotypes, because suppressing its expression in adults has no effect on receptivity. *Abd-B*, a gene in the bithorax complex, determines the fate of the posterior segments of the fly, including the terminal segments of the nervous system called the abdominal ganglion. *Abd-B* had not previously been connected to female sexual behaviour, despite being associated with male

sexual behavior through the determination of a male-specific abdominal muscle called the muscle of Lawrence [9] (itself controlled by *fruitless* [10], a male sexual behavior determinant [1]), as well as in the proper functioning of male seminal fluids [11].

The involvement of *Abd-B* in female behavior recalls an often overlooked fact: genes that influence adult behavior may do so by controlling the development of the nervous system and do not necessarily have an ongoing physiological function in the adult [12]. The work of Bussell *et al.* [2] elegantly shows that artificial physiological activation of *Abd-B* neurons in adult females induces increased pausing. Thus, even though *Abd-B* gene expression is no longer required in adults, these neurons have been determined to function in pausing, perhaps through *Abd-B* laying down their development to be a circuit element of female receptivity.

While females may appear coy during courtship, their acceptance or rejection of a male depends on integration of all elements of male courtship [5]. How do they perform this integration and what happens between sensing of a courtship cue and the decision to initiate mating? In a study from the Baker lab published in *Neuron*, Zhou *et al.* [3] reasoned that the fly brain, because it receives input from all senses, should contain the higher processing centre for integrating courtship cues and therefore be the decision-centre of virgin female receptivity. Zhou *et al.* [3] took advantage of the influence of neurons expressing the *doublesex* (*dsx*) gene on female receptivity. The Goodwin lab had previously reported that *dsx* is expressed in female neurons and that artificially silencing these neurons reduces female receptivity [13]. Expression of *dsx* in the female brain is restricted to three clusters in the dorsal protocerebrum called pC1, pC2 and pCd. Using an intersectional approach (see Liu and Yang [14] for details), Zhou *et al.* [3] managed to reproducibly gain control over each of these three clusters allowing them to artificially activate or silence these neurons and looked for changes in receptivity.

In this way, Zhou *et al.* [3] identified pC1 and pCd as important neuronal clusters sufficient to modulate female receptivity. Having obtained tools to gain access to those higher processing neurons, they developed a method to monitor their physiological activity in a live female exposed to a male excitatory pheromone called cis-vaccenyl acetate (cVA) [15] and to a playback of a courtship song. pCd and pC1 neurons increased their calcium response to cVA, while pC1 neurons also responded to male courtship song. Concomitant exposure of cVA enhanced the response of pC1 neurons to song, which may indicate that these neuronal clusters integrate these two signals. Both pCd and pC1 neurons have post-synaptic connections in the lateral- and superior protocerebrum, where they may make contact with female-specific third order neurons that receive cVA input [16]. pCd neurons have pre-synaptic connections in the suboesophageal zone, where a set of second-order olfactory neurons required for female receptivity also terminates [17].

Intriguingly, Bussell *et al.* [2] also demonstrated that a subset of *Abd-B*-expressing neurons in the abdominal ganglion, those important for receptivity and female pausing, project to the brain in the suboesophageal zone, lateral neurons and superior neuropils. That they send post-synaptic projections to the same sites where pC1 and pCd *dsx* neurons arborize indicate that pC1 and pCd may be a hub for multisensory integration, a neuronal correlate of the behavioral observation that female receptivity is the result of the integration of multiple male signals [5].

The final piece of the receptivity puzzle is how females transition from receptive virgin females to an unreceptive post-mating state. The male cue that induces this transition is a peptide transferred during mating called the Sex-peptide (Sp) [5]. Sensory neurons detecting Sp are located in the wall of the uterus, close to where the ejaculate is stored [18–20]. These neurons terminate in the abdominal ganglion, so the same integration problem arises: how is this mating status signal transferred to higher processing centres?

Again using an intersectional approach, the Dickson group [4] identified interneurons called SAG that affect female post-mating receptivity located in the abdominal ganglion where they make synaptic contact with Sp receptor neurons. In recent years, several papers have investigated the neuronal circuitry underlying post-mating receptivity [5]. These elegant studies succeeded in identifying single neurons or small populations of neurons that are part of this circuitry. The ultimate goal of identifying an element of a circuit is, however, to connect those different single neurons that must act in concert to produce behavior.

The work of Feng *et al.* [4] represents a breakthrough and a *tour de force* in the study of circuitry and female receptivity: they demonstrate that SAG neurons are indeed synaptic partners of Sp receptor by developing an *ex vivo* preparation in which they can directly patch and record from SAG neurons post-synaptic to Sp receptor neurons. By doing so they no longer study single elements of a circuit but are getting at the circuit itself. Interestingly, SAG neurons send projections to the brain to areas innervated by pC1 and pCd neurons. Along with courtship cues these brain areas might permit an additional cue to be integrated: that the female has successfully mated.

These three papers [2–4] represent what neurogenetics of female reproductive behavior is about: genes with a female receptivity phenotype provide a gateway into the nervous system allowing us to peer at the developmental and physiological events that permit interconnected neurons to control behavior. Together, the three studies identify neuronal populations in the abdominal ganglion and the central brain that control receptivity and call attention to how behaviors develop and are modified through an individual's lifetime. Thanks to these studies the neuronal substrate for female behavior is no longer confined to a single neuronal population or a single brain *locus*. Instead they indicate that female receptivity involves a circuit that includes remote but interconnected parts of the nervous system that arise through development and are modified by sexual experience.

References

1. Yamamoto, D., and Koganezawa, M. (2013). Genes and circuits of courtship behaviour in Drosophila males. Nat. Rev. Neurosci. *14*, 681–692.
2. Bussell, J.J., Yapici, N., Zhang, S.X., Dickson, B.J., and Vosshall, L.B. (2014). Abdominal-B neurons control Drosophila virgin female receptivity. Curr. Biol. *24*, 1584–1595.
3. Zhou, C., Pan, Y., Robinett, C.C., Meissner, G.W., and Baker, B.S. (2014). Central brain neurons expressing doublesex regulate female receptivity in Drosophila. Neuron *83*, 149–163.
4. Feng, K., Palfreyman, M.T., Häsemeyer, M., Talsma, A., and Dickson, B.J. (2014). Ascending SAG neurons control sexual

receptivity of Drosophila females. Neuron *83*, 135–148.
5. Laturney, M., and Billeter, J.-C. (2014). Neurogenetics of female reproductive behaviors in Drosophila melanogaster. Adv. Genet. *85*, 1–108.
6. Fabre, C.C.G., Hedwig, B., Conduit, G., Lawrence, P.A., Goodwin, S.F., and Casal, J. (2012). Substrate-borne vibratory communication during courtship in Drosophila melanogaster. Curr. Biol. *22*, 2180–2185.
7. Billeter, J.-C., Atallah, J., Krupp, J.J., Millar, J.G., and Levine, J.D. (2009). Specialized cells tag sexual and species identity in Drosophila melanogaster. Nature *461*, 987–991.
8. Trott, A.R., Donelson, N.C., Griffith, L.C., and Ejima, A. (2012). Song choice is modulated by female movement in Drosophila males. PLoS ONE *7*, e46025.
9. Lawrence, P.A., and Johnston, P. (1986). The muscle pattern of a segment of Drosophila may be determined by neurons and not by contributing myoblasts. Cell *45*, 505–513.
10. Gailey, D.A., Taylor, B.J., and Hall, J.C. (1991). Elements of the fruitless locus regulate development of the muscle of Lawrence, a male-specific structure in the abdomen of Drosophila melanogaster adults. Development *113*, 879–890.
11. Gligorov, D., Sitnik, J.L., Maeda, R.K., Wolfner, M.F., and Karch, F. (2013). A novel function for the Hox gene Abd-B in the male accessory gland regulates the long-term female post-mating response in Drosophila. PLoS Genet. *9*, e1003395.
12. Bate, M. (1998). Making sense of behavior. Int. J. Dev. Biol. *42*, 507–509.
13. Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie, S., and Goodwin, S.F. (2010). Control of sexual differentiation and behavior by the doublesex gene in Drosophila melanogaster. Nat. Neurosci. *13*, 458–466.
14. Liu, Y., and Yang, C. (2014). Unveiling the Secrets to Her Heart. Neuron *83*, 3–5.
15. Kurtovic, A., Widmer, A., and Dickson, B.J. (2007). A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature *446*, 542–546.
16. Kohl, J., Ostrovsky, A.D., Frechter, S., and Jefferis, G.S.X.E. (2013). A bidirectional circuit switch reroutes pheromone signals in male and female brains. Cell *155*, 1610–1623.
17. Sakurai, A., Koganezawa, M., Yasunaga, K.-I., Emoto, K., and Yamamoto, D. (2013). Select interneuron clusters determine female sexual receptivity in Drosophila. Nat. Commun. *4*, 1825.
18. Hasemeyer, M., Yapici, N., Heberlein, U., and Dickson, B.J. (2009). Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. Neuron *61*, 511–518.
19. Yang, C.-H., Rumpf, S., Xiang, Y., Gordon, M.D., Song, W., Jan, L.Y., and Jan, Y.-N. (2009). Control of the postmating behavioral switch in Drosophila females by internal sensory neurons. Neuron *61*, 519–526.
20. Rezával, C., Pavlou, H.J., Dornan, A.J., Chan, Y.-B., Kravitz, E.A., and Goodwin, S.F. (2012). Neural circuitry underlying Drosophila female postmating behavioral responses. Curr. Biol. *22*, 1155–1165.

[1]Behavioural Biology, Centre for Behaviour and Neurosciences, PO Box 11103, University of Groningen, Groningen, 9700 CC, The Netherlands. [2]Department of Biology, University of Toronto at Mississauga, 3359 Mississauga Road, Mississauga, ON, L5L 1C6, Canada.
E-mail: j.c.billeter@rug.nl, jlevmail@gmail.com

http://dx.doi.org/10.1016/j.cub.2014.07.052